**953-Plat**

**Label-Free Determination of Compositional Fluctuations and Macroscopic Phase Separation in a Ternary Lipid Mixture**

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Reports on phase coexistence regimes and directions of tie lines of ternary lipid mixtures are often controversial. The origin of these controversies is typically the experimental window of the applied experimental techniques. Additional complications arrive due to putative influences of labels on the phase behavior. Therefore, we combined small- and wide-angle x-ray scattering, differential scanning calorimetry and attenuated total reflection-Fourier transform infrared spectroscopy to probe the stability and physical properties of coexisting domains under label-free conditions. The capabilities of this combination are demonstrated on a model system composed of palmitoyl oleoyl phosphatidylcholine, sphingomyelin and ceramide. This mixture mimics sphingomyelinase activity on biological membranes. We found compositional fluctuations (unstable microscopic domains) in the absence of ceramide and macroscopically separated fluid and gel phases upon the addition of ceramide. Additionally, we observed broad phase transitional regions in the presence of ceramide, where also phase fluctuations occurred. Results are compared to a previously reported phase diagram and discussed in relation to the biological activity of sphingomyelinase. Our study demonstrates the necessity of applying a mix of experimental techniques to probe local/global structural, as well as fast/slow motional properties in complex lipid mixtures.

**954-Plat**

**Area Deformation of Membranes from the Perspective of $^2$H NMR and X-ray Scattering**

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We address the hypothesis that functions of cellular membranes are affected by non-specific lipid-protein interactions due to bilayer material properties that depend on both pressure and temperature [1]. Changes of either pressure or temperature cause lipid bilayer deformations that are quantified by $^2$H NMR and X-ray scattering for membranes under osmotic stress. We present measurements of membrane structural parameters such as bilayer thickness and the area per lipid by employing a mean-torque analysis [2-3] of $^2$H solid-state NMR results together with X-ray scattering data. The $^2$H NMR experiments for both hydration pressure (low water content) and osmotic pressure (with poly(ethyleneglycol)) show that the segmental order parameters ($S_{CD}$) of DMPC approach very large values of $\approx$ 0.35 in the liquid-crystalline state. These two pressures are thermodynamically equivalent, because the change in chemical potential when transferring water from the interlamellar space to the bulk water phase corresponds to the induced pressure, as experimentally verified by NMR measurements [4]. By considering the equations of state at thermal equilibrium, we extend this approach to address the correspondence between osmotic pressure and hydrostatic pressure. Area per lipid measured using both NMR and X-ray measurements provides a thermodynamic parameter that quantifies membrane deformations [2]. Combined analysis of NMR and X-ray allows us to further test our understanding of dehydration and osmotic stress-induced membrane deformation. We conclude that solid-state $^2$H NMR spectroscopy and X-ray scattering together with bilayer membrane stress techniques are important tools for understanding of the mechanism of pressure sensitivity of membrane proteins. [1] A.V. Botelho *et al*. (2006) *Biophys. J.***91**, 4464-4477. [2] H.I. Petrache *et al*. (2000) *Biophys. J.***79**, 3172-3192. [3] H.I. Petrache *et al*. (2001) *JACS* **123**, 12611-12622. [4] K.J. Mallikarjunaiah *et al*. *Biophys. J.*(in press).

**955-Plat**

**Curvature-Driven Dynamic Sorting of Lipids and Proteins in Membrane Tubes: The Race Between Convection and Diffusion**

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Conventional models of multi-component membranes usually assume the lipid velocity induced by membranes shape change is small. Hence, The Peclet number is small and convection is negligible compared to diffusion. However, the situation is dramatically different if the deformation of membranes is big and fast. In this paper, we report striking effects of convection when membrane tubes are pulled out from liquid ordered (Lo) phase with various pulling speeds. The competition between curvature-driven lipid sorting and the pulling-induced convection leads to new behavior. If the pulling speed is very big, the diffusion process is much slower than the pulling process. The lipids are "frozen" on the surface and diffusion can be neglected. The whole membrane is in Lo phase. If the pulling speed is very small, the membrane shape change slowly and lipids have enough time to diffuse. The high curvature of the membrane tube will drive the formation of the liquid disordered (Ld) phase. If the pulling speed is comparable to the diffusion speed, some interesting phenomena appear. For example, one Ld domain is nucleated around the neck region and grows larger with time for relatively low pulling speed. If the pulling speed is a little higher, multiple Ld domains can be nucleated and pulled out from the neck region. Finally, experiments are designed to verify the predictions.

**956-Plat**

**Contribution of S4 Charges to Gating Mechanism in Hv Channels**

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Voltage-gated proton ($H_V$) channels have been shown to play an essential role in immune system function. They are homologous to the voltage-sensing domain (VSD) of voltage-gated potassium (Kv) channels. In contrast to the tetramer structure of Kv channels, we found that Hv channels are dimers with just two S4 segments. Recently, we showed that the total effective gating charges are $5.9 \pm 0.4 e_0$ in the dimer and $2.7 \pm 0.1 e_0$ in the monomer and that S4 movement (containing 3 arginines) precedes channel opening. However, which specific arginine from S4 segment contributes to the effective gating charge is still unknown. To answer this question, we replaced each arginine residue with asparagines separately in *Ciona intestinalis* Hv channels. Patch-clamp recordings in *Xenopus* oocytes showed a dramatic decrease of total gating charges when measured by the limiting slope method: 1.9, 2.9 and 2.2 $e_0$ for R255, R258 and R261, respectively. In addition, our cysteine accessibility measurements are consistent with an outward movement of these three S4 charges during channel opening. According to our findings, we conclude that the S4 segment moves and functions as the voltage sensor and all S4 charges contribute to voltage gating in Hv channels.

**957-Plat**

**Ionization State of Phosphatidylinositol-4,5-Bisphosphate in the Presence of Lipids with Hydrogen Bond Donor Capabilities**

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Phosphatidylinositol 4,5-bisphosphate [PI(4,5)P2] is a minor component in the cell membrane, but has very important roles in a broad range of cell functions. Many proteins have binding domains that bind specifically to PI(4,5)P2. While hydrogen bond interactions play a pivotal role in these interactions, electrostatic interactions strongly contribute to any protein/PI(4,5)P2 binding event. In addition, the lateral distribution of PI(4,5)P2 within the bilayer is strongly dependent on its ionization state. Although the ionization state of PI(4,5)P2 has been examined in previous research, there has been no data on the ionization properties of PI(4,5)P2 in more complex lipid mixtures that contain lipids capable to donate hydrogen for bond formation with PI(4,5)P2. Here we investigate the ionization state of PI(4,5)P2 in mixtures of three or more lipids. Using MAS solid state 31P-NMR, we examined the ionization state of PI(4,5)P2 in multilamellar vesicles composed of DOPC and DOPE (70% DOPC, 25% DOPE, 5% PI(4,5)P2) for pH values between 4 and 10. In comparison to the binary PC/PI(4,5)P2 mixture (Kooijman et al. Biochemistry 48 (2008) 9360), the titration curve is shifted to lower pH values, indicating an increased deprotonation of the PI(4,5)P2 phosphomonoester groups. In additioin, the titration curve has a complex shape, suggesting a pronounced interaction between DOPE and PI(4,5)P2. We are currently examining the ionization properties of PI(4,5)P2 in the presence of phosphatidylinositol (PI) since it is likely that these two lipids are co-localized in the plasma membrane. Our NMR studies are being complemented by GUV studies of ternary lipid systems. For example, it is found that PE has a strong effect on the morphology (domain structure) of PI(4,5)P2 containing lipid systems.

**958-Plat**

**Structure and Dynamics of Lipid-Modified Antimicrobial Peptides in Anionic and Zwitterionic Membranes Investigated by Solid-State NMR**

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The increasing prevalence of antibiotic resistant strains of bacteria necessitates the development of new antibiotic drugs, preferably operating via novel pathways to avoid cross-resistance with drugs already in use. The group of Shai and coworkers has recently proposed a new set of very short lipid-modified

antimicrobial peptides showing promising properties for possible application. We investigated two of these peptides, C16-KGGK and C16-KAAK in two different lipid environments, one more resembling mammalian membranes (POPC) and the other closer to bacterial membranes (POPE/POPG 2:1). Investigations were conducted on powder-type samples at a lipid/peptide ratio of 9:1 and a temperature of 303K. First, the host membranes were investigated using $^{31}$P solid-state NMR clearly showing no influence of the peptides on the lamellar membrane phase state. Information about the chain dynamics and membrane packing properties was obtained using $^{2}$H solid-state NMR. Order parameters of the lipids were slightly reduced upon addition of the peptide. However, the lipid modifications generally exhibit higher order parameters than the surrounding lipids meaning that the length of the peptide lipid modifications is larger than that of the lipid acyl chains. This is in agreement with paramagnetic relaxation enhancement data exhibiting interactions between the amino acids and spin-labeled phospholipids suggesting a peptide backbone location in the headgroup region of the membrane. The dynamics of the lipid modifications were investigated by means of $^{2}$H $R_{1Z}$ relaxation rates. While other lipid-modified peptides exhibit square law plots that are bent the ones obtained for the antimicrobial peptides are linear and resemble that of saturated lipids. Therefore the lipid modifications of the antimicrobial peptides are less flexible and longer than that of other lipid-modified peptides allowing the peptide backbone to be located in the lipid headgroup region.

**959-Plat**

**A Comparative Molecular Dynamics Study of the Effect of Alpha-Tocopherol and Cholesterol on Phospholipid Bilayers having Different Levels of Acyl Chain Unsaturation**

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Cholesterol and alpha-tocopherol (vitamin E) are important constituents of cell membranes having similar molecular shapes and sizes. These two molecules, however, exhibit very different properties in membranes and are thought to play different roles in the biology of the cell. Cholesterol is found in high concentrations in membranes, especially those composed of saturated phospholipids such as 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), while recent experiments suggest that alpha-tocopherol, which is typically found in much smaller concentrations, preferentially accumulates in membranes containing highly polyunsaturated lipids such as 1-stearoyl-2-docosahexaenoyl-sn-glycero-3-phosphocholine (SDPC). Here we examine the structural and dynamic properties of alpha-tocopherol and cholesterol and their effects on phospholipid bilayers using molecular dynamics (MD) computer simulation methods. Six long simulations (hundreds of nanosecond each) have been carried out, one for each neat lipid (DPPC and SDPC), and one for each solute in each lipid. The dramatically different effect on membrane properties that is observed in the simulation is explained in terms of unique solute-lipid interactions arising from polyunsaturation of the lipid acyl chains.

# PLATFORM S: Fluorescence Spectroscopy

**960-Plat**

**Tryptophan Fluorescence Modulated by Histidine Quenching During Folding of Small Alpha Helical Peptides: Distance and Solvation Effects**

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Tryptophan (Trp) fluorescence has been extensively used to study the folding kinetics of small alpha helical proteins including: the villin headpiece N68H (1yrf), villin headpiece K65(NLE) N68H K70(NLE) (2f4k) and a peptide Ac-Trp-$(Ala)_3$-$His^+$-$NH_2$(WH5). In these three cases a histidine (His) has been placed in an alpha helical section of the protein four amino acids away from Trp, so that His will quench the fluorescence of Trp as the protein folds. $His^+$ quenches the fluorescence of free Trp in solution at a diffusion limited rate by electron transfer from the Trp ring to the $His^+$ ring. The rate of electron transfer is known to fall off exponentially due to the exponential decay of the electronic coupling $V_{el}$ with distance. In this work we show that this decay also depends on how the energy gap is affected by distance. Solvation around the imidazole cation at large distances increases the energy gap, since waters around the cation point their dipoles towards the ring, destabilizing the charge transfer state. At short distances, the decreased water accessibility reduces the destabilization of the charge transfer state, enhancing electron transfer from the Trp ring to the $His^+$ ring. This is seen in five different proteins: 1yrf, 2f4k, barnase (1a2p), T4-lysozyme (1lyd) and WH5. We also examine the ability of Trp fluorescence to determine folding rates for 1yrf, 2f4k and WH5 by using QM/MM simulations to determine the electron transfer rates in the folded and unfolded states.

**961-Plat**

**Structural Heterogeneity and Quantitative Single Molecule FRET Efficiency Distributions of Polyproline Through a Hybrid Atomistic Simulation and Monte Carlo Approach**

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Förster Resonance Energy Transfer (FRET) experiments allow to probe molecular distances via the distance dependent energy transfer efficiency from an excited donor dye to its acceptor counterpart. In single molecule settings, not only average distances, but also distance distributions or even fluctuations can be probed, providing a powerful tool to study structural changes in biomolecules. However, the measured energy transfer efficiency depends not only on the distance between the dyes, but also on their mutual orientation, which is typically inaccessible to experiments. Thus, assumptions on the orientation distributions and averages usually have to be employed, severely limit the accuracy of the distance distributions extracted from FRET experiments.

Here, we demonstrate that by combining FRET experiments with the mutual dye orientation statistics obtained from Molecular Dynamics (MD) simulations, improved estimates of the distance distributions can be obtained. From the time-dependent mutual orientations, the FRET efficiency is calculated and the statistics of individual photon absorption, FRET transfer, and photon emission events is determined from subsequent Monte Carlo (MC) simulations. All recorded emission events are then collected to bursts from which efficiencies are calculated in close resemblance to the actual FRET experiment. For several test systems, we demonstrate the feasibility of our approach by direct comparison to experimental data.

In particular, we have studied a poly-proline chain with attached Alexa488 and Alexa594 dyes. Calculated efficiency distributions from our simulations agreed with the experimental findings and identify the presence of cis-isomers as one source of the experimentally observed heterogeneity. This result demonstrates that dye orientations from MD simulaions, combined with MC photon generation, can indeed be used to improve the accuracy of distance distribution reconstruction from experimental FRET efficiencies.

**962-Plat**

**The Photochemistry of Bacteriophytochrome: Key to its Use as a Deep-Tissue Fluorescence Probe**

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Bacteriophytochromes (Bphs) are red-light photoreceptors that regulate a variety of bacterial responses. Their photosensory core consists of PAS, GAF and PHY domains and covalently binds biliverdin (BV). The Bph light activation mechanism involves isomerization around the BV C15=C16 double bond, resulting in a flip of its D-ring. In an important recent development, PAS-GAF variants were engineered for use as near-infrared fluorescent markers in mammalian tissues (Shu et al. Science 2009). Here, we report the photochemistry of two Bphs from Rps. palustris, RpBphP2 (P2) and RpBphP3 (P3) that have distinct photoconversion and fluorescence properties. We applied ultrafast spectroscopy on P3 and P2 PAS-GAF proteins and the P3 D216A and P2 D202A PAS-GAF-PHY proteins. In these mutants a conserved aspartate which connects BV with the PHY domain through extensive hydrogen-bond networks, was replaced by alanine. The excited-state lifetime of P3 and P2 PAS-GAF was significantly larger than their PAS-GAF-PHY counterparts. Mutation of the conserved Asp to Ala in PAS-GAF-PHY had a similar but larger effect. In particular, the fluorescence quantum yield of the P3 D216A mutant was 0.066, higher than that of wild type P3 (0.043) and similar to the engineered Bph of Shu et al. We conclude that elimination of a key hydrogen-bond interaction between Asp and a conserved Arg in the PHY domain is responsible for the excited-state lifetime increase. H/D exchange resulted in a 1.5 - 1.7 fold increase of excited-state lifetime. The results are rationalized with a reaction model where excited-state deactivation of BV proceeds via excited-state proton transfer from the BV pyrrole nitrogens to the backbone of the conserved Asp or a bound water. This work may aid in rational structure- and mechanism-based conversion of P3 and other BPhs into efficient near-IR fluorescent markers.